Bioprocess and Biosystems Engineering manuscript No.
(will be inserted by the editor)

Life cycle greenhouse gas emissions of microalgal fuel from thin-layer cascades

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Received: date / Accepted: date

Abstract Thin-layer cascades (TLCs) enable algae cultivation at high cell densities, thus increasing biomass yields and facilitating the harvest process. This makes them a promising technology for industrial-scale algal fuel production. We use Life Cycle Assessment (LCA) to calculate the greenhouse gas (GHG) emissions of aviation fuel produced using algal biomass from TLCs. We find that the impact (81 g CO_2e per MJ) is lower than that of fuel from algal biomass cultivated in open race way ponds (94 g CO_2e). However, neither of the two cultivation systems achieve sufficient GHG savings for compliance with the Renewable Energy Directive II. Seawater desalination in particular dominates the TLC impact, indicating a trade-off between carbon- and water footprint. In both cultivation systems, power for mixing and fertilizer sup-

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The authors thank Christoph Falter, Valentin Batteiger, and Andreas Sizmann for providing useful advice in preparation of the manuscript. This work was supported financially by the Bavarian Ministry of Economic Affairs, Regional Development and Energy via grant number LABAY74C. This is a preprint of an article published in Bioprocess and Biosystems Engineering. The final authenticated version is available online at: `https://doi.org/10.1007/s00449-021-02612-9`

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ply further add significant impacts. There is uncertainty in the correlation between mixing power and oil yield, which should be investigated by future experimental studies.

Keywords life cycle assessment · greenhouse gases · microalgae · fuel · thin-layer cascade · cultivation system

1 Introduction

In 2018, the global transport of passengers and goods was responsible for 8.0 Gt of CO_2 emissions (24% of total fuel-related emissions) [1]. Avoiding these emissions is the aim of several frameworks to which the international community has committed [2, 3]. Biofuels will play an important role in these efforts, despite the advent of batteries and fuel cells, especially in sectors like long-haul aviation, which require energy-dense fuels. To meet the demand of these sectors while avoiding land competition with the food & feed sector, low land-use change-risk biofuel-feedstocks are needed. Microalgae offer several advantages in this regard, i.e. the possibility to use marginal lands for their cultivation and their high theoretical biomass yield [4]. Yet, there are doubts whether algal fuels can achieve the greenhouse gas emission (GHG) reductions necessary to comply with existing regulations, such as the Renewable Energy Directive II in the EU (RED II) [5] and the Renewable Fuel Standard in the US (RFS) [6]. In a meta-analysis comprising 69 Life Cycle Assessments (LCAs) of renewable algal diesel, Tu et al. [7] found that only 17 (25%) of the reported pathways comply with the RFS (50% GHG reduction compared to 2005 baseline diesel). The RED II mandates even higher GHG savings of 65% for biofuel producers starting operation after 1 January 2021 [5]. Needless to say, the algal fuel community is facing a challenge. Several studies have identified the cultivation stage as a bottleneck on the way towards economically- and environmentally feasible algal fuel production [8, 9, 10, 11]. Alternative cultivation technologies could reduce the cultivation impact, enabling the production of affordable, regulation-compliant algal fuel. One such alternative is proposed by Doucha and Lívanský [12]: Comparing sloping thin-layer cascades (TLCs) to conventional open raceway ponds (ORPs), they find that the former require less power, water, and CO_2 per unit biomass produced. Although TLCs date back to the 1950s, they have received relatively little attention to date [13, 14]. The existing literature on TLCs is mainly focused on technical aspects, such as the optimization of operation conditions and the measurement of culture parameters [15, 16, 17, 18, 19, 20, 21, 22]. Furthermore, a few techno-economic assessments have been published [12, 23, 24]. To the authors' knowledge, no LCA of algae cultivation in TLCs has been conducted so far - a gap we wish to close with this study.

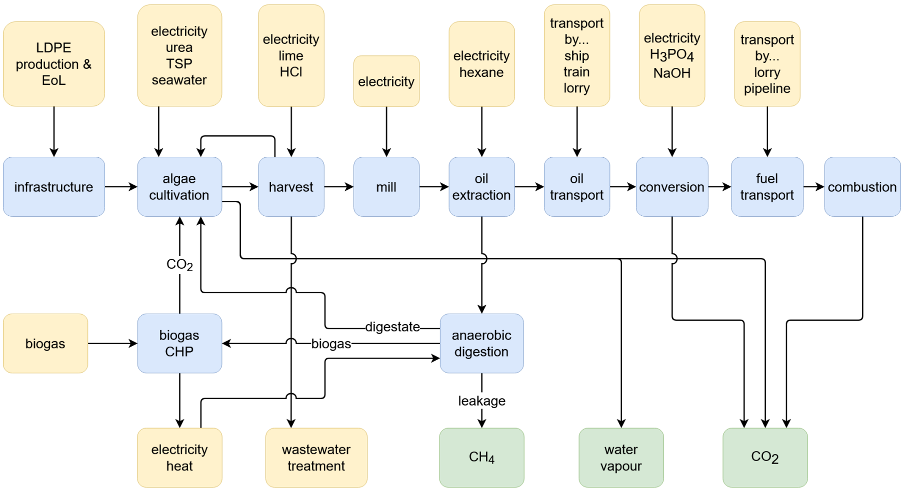

Fig. 1 Algae fuel production pathway. Boxes represent process steps. Colors distinguish process types: foreground database (blue), background database (yellow), biosphere (green)

2 Method

Life Cycle Assessment (LCA) is used to calculate the Global Warming Potential (GWP 100) of the TLC algae fuel pathway producing 1 MJ (lower heating value) of fuel (functional unit). The life cycle comprises the following stages: a biogas-fired combined heat and power (CHP) plant provides CO_2 to the algae cultivation facility where algae grow autotrophically in TLCs, the biomass is harvested, converted into fuel, residual biomass is valorized energetically, fuel is transported and combusted (Figure 1). Models of the individual stages are based on engineering first principles and are tuned to parameters from the literature and from expert interviews. Models from the ecoinvent 3.6 APOS database are used for background activities, such as electricity generation and fertilizer production [25]. The impact assessment is conducted in Brightway2 using the ILCD 2.0 2018 midpoint, climate change total method [26]. Apart from fuel, the studied pathway produces electricity and heat. This multifunctionality is resolved by subtractive system expansion. The TLC pathway is compared to two other options, namely conventional petroleum-based fuel production and algal fuel production using ORPs in the cultivation stage. The algae pathways are presented briefly in the following. For more detailed reference, we refer to the Excel spreadsheets available in the supporting information.

3 Model

3.1 CO_2 source

CO_2 from a biogas-fired CHP plant serves as the primary carbon source for autotrophic microalgae cultivation. The model is based on ecoinvent activity *heat and power co-generation, biogas, gas engine, ES* and has been edited to a) eliminate CO_2 emissions to the atmosphere and b) implement an energy penalty of 8% for extraction and compression of CO_2 from the flue gas, based on Lively et al. [27]. The CHP plant then produces 5.8 MJ of useful heat and 4.1 MJ of electricity for each kg of CO_2 captured.

3.2 Cultivation

In their modern form, thin-layer cascades consist of a thin film, few mm thick, flowing down an inclined plane driven by gravity. Thanks to the short light path and the large surface-to-volume ratio of the thin film, TLCs can maintain high growth rates up until cell concentrations of 40 – 50 g_{DW}/L [19, 21]. Seasonal productivity typically ranges between 22 – 25 $g_{DW}/(m^2 d)$ [12]. For our study, we consider a hypothetical cultivation plant in a coastal area in Spain, which is operated 8 months per year. A seasonal productivity of 25 $g_{DW}/(m^2 d)$ is assumed at an oil content of 30 wt-%. A cultivation area of 100 ha will then yield 1 800 t of algae oil per year.

A pump continuously circulates the cultivation medium from the bottom to the top of the plane. Excluding friction losses, the pump power demand per unit area is proportional to the plane inclination I, the layer thickness h, the flow velocity u, the gravity constant $g = 9.81\ m/s^2$, the medium density $\rho \approx 1\ 000\ kg/m^3$, and the inverse of the pump efficiency η_p [18]:

$$\frac{P}{A} = \frac{I\ h\ u\ g\ \rho}{\eta_p} \tag{1}$$

Using values from Doucha and Lívanský [18] for I (1.7%), h (6 mm), u (0.6 m/s), and assuming a pump efficiency of 80%, the power demand amounts to 0.75 W/m^2. The pump is operated only during the day (12 hours). At night, the medium is stored in a retention tank where it is mildly aerated. Doucha and Lívanský [12] approximate the power demand for aeration at 40% of the demand for pumping. The total power demand is thus 12.6 $Wh/(d\ m^2)$ or 0.50 Wh/g_{DW}. Note that this is a lower-bound estimate, as additional energy loss mechanisms such as friction in pipes and fittings have been neglected.

Apart from power, nutrients are needed in the cultivation stage to maintain algae growth. We follow the approach described by Geider and La Roche [28] to derive the demand for elements C, N, and P from the macro-molecular biomass composition. The latter is presumed to be (by weight) 35% proteins, 30% lipids, 10% phospholipids, 20% carbohydrates, and 5% nucleic acids. Part of the elemental demand is satisfied by recycling digestate and flue gas from the residue valorization step (30% of the C-demand and 48% of the N- and P-demand), the rest is fulfilled by supplementation of CO_2, urea, and triple superphosphate (TSP). It is assumed that, due to technical limitations, 25% of the supplied CO_2 are lost by out-gassing and 15% of the supplied N in urea are lost by other mechanisms. The net nutrient input is then 1.9 kg CO_2, 0.17 kg urea, and 0.035 kg TSP per $\mathrm{kg_{DW}}$ biomass.

Lastly, water replenishment is needed to compensate losses from evaporation and technical blow-down. Guieysse et al. [29] estimate the evaporation loss rate for a Mediterranean climate at 1.3 $\mathrm{m^3/(m^2\ a)}$. Assuming that algae are cultivated in seawater to reduce the freshwater footprint, evaporation will cause an increase in salt concentration. To prevent inhibiting conditions, both water and salt-flows must be balanced. Conducting a mass balance for both components yields:

$$\dot{m}_{\text{fresh}} = \dot{m}_{\text{evap}} + \dot{m}_{\text{harvest}}\,(1-R)\left(1-\frac{c_{\text{culture}}}{c_{\text{sea}}}\right) \tag{2}$$

$$\dot{m}_{\text{sea}} = \dot{m}_{\text{harvest}}\,(1-R)\,\frac{c_{\text{culture}}}{c_{\text{sea}}} \tag{3}$$

where $\dot{m}_{\text{fresh}}$ is the demand for freshwater, $\dot{m}_{\text{sea}}$ is the demand for seawater, $\dot{m}_{\text{evap}}$ is the evaporation rate, $\dot{m}_{\text{harvest}}$ is the amount of water in the harvest, R is the fraction of water returned to the reactor after the harvest, c_{culture} is the salt concentration in the reactor and c_{sea} is the concentration of salt in the seawater. The flow rates $\dot{m}_i$ here are normalized by the biomass production rate of the reactor, yielding units of gram water per gram biomass dry weight.

Note that if the salt concentration in the reactor is higher than in the seawater ($\frac{c_{\text{culture}}}{c_{\text{sea}}} > 1$), the second term in eq. 2 turns negative, meaning that freshwater consumption can be reduced by increasing the blow-down. In other words: The use of halotolerant species reduces the freshwater demand (although at the cost of an increased seawater demand). The salt concentration at which freshwater demand is zero is given by:

$$c^0_{\text{culture}} = c_{\text{sea}}\left(1+\frac{\dot{m}_{\text{evap}}}{\dot{m}_{\text{harvest}}\,(1-R)}\right) \tag{4}$$

For TLCs, the biomass concentration at the time of harvest is relatively high (here 20 g/L). This in turn means that relatively little water is removed during the harvest process (50 $\mathrm{g/g_{DW}}$) compared to evaporation (220 $\mathrm{g/g_{DW}}$). According to eq. 4, the salt concentration would have to be at least 19% to avoid freshwater use (for $c_{\text{sea}} = 3.5\%$ and $R = 0$). Such high concentrations would require adapted algae strains and it is questionable whether these would be suitable for industrial-scale biofuel production. For most use cases, freshwater will be necessary for TLC cultivation. This is in contrast to ORPs, in which the biomass concentration is usually low, making harvest water blow-down an effective mechanism for salt removal. Nevertheless, we assume that the TLC is operated at a slightly elevated salt concentration of 5.3% to reduce freshwater demand.

All water used in the cultivation stage is supplied by a pipeline from the neighboring sea. Freshwater is provided by re-routing part of the seawater feed through a reverse osmosis plant. Seawater is used without pre-treatment. The energy demand for water transport is given by eq. 5 and depends on the total transport demand $\dot{m} = 260$ g/g$_{\mathrm{DW}}$, the gravity constant $g = 9.81\mathrm{m/s}^2$, the pump efficiency η_p and the head loss Δh. The latter will depend on the distance to the sea, the height difference between inlet and outlet of the pipeline, as well as friction losses in between. Without a concrete design at hand, we assume an arbitrary head loss of 60 m. The power demand then amounts to 0.054 Wh/g$_{\mathrm{DW}}$. Note that this number is one order of magnitude smaller than the demand for culture circulation.

$$P = \frac{\dot{m}\, g\, \Delta h}{\eta_p} \tag{5}$$

ORP cultivation requirements are similar to TLC requirements except for certain points highlighted in the following. ORPs are deeper (here 30 cm) than TLCs (here 6 mm) and algae cells in deeper layers receive less sun light than those at the surface. The lower average sun exposure leads to lower biomass yields on the order of 15 g$_{\mathrm{DW}}$/(m^{2}d) and lower harvest cell densities around 0.5 g$_{\mathrm{DW}}$/L. Because, in comparison to TLCs, more area is needed for the same biomass production rate, specific evaporation is increased (360 g/g$_{\mathrm{DW}}$). Furthermore, the water portion in the harvested medium is significantly higher (2 000 g/g$_{\mathrm{DW}}$). Operating the system at a blow-down ratio of 36% ($R = 0.64$) and at a slightly elevated salt concentration $c^0_{\mathrm{culture}} = 5.3\%$ eliminates freshwater demand and yields a saltwater demand of 1 100 g/g$_{\mathrm{DW}}$. The power demand for water transport is then 0.22 Wh/g$_{\mathrm{DW}}$. ORPs are typically mixed by paddle wheels to enable homogeneous sun exposure and to facilitate oxygen removal. Their energy demand depends on several factors, such as the mixing velocity and the depth of the pond [30]. Here, a mixing power requirement of 0.4 W/m^2 (= 0.48 Wh/g$_{\mathrm{DW}}$) is assumed [30]. Nutrient demand per unit biomass is identical for ORP and TLC cultivation, assuming identical biomass composition and nutrient utilization ratios.

For the construction phase, we assume that both reactor types can be cost-effectively implemented by spreading LDPE pond liners over compacted sand. Furthermore, a pipeline is built to deliver seawater to the cultivation units. End of life for both liners and pipeline is accounted for using ecoinvent markets.

3.3 Harvest

Harvest procedures differ between ORP and TLC systems due to different biomass concentrations at the time of harvest. For TLCs, the culture medium is centrifuged until a biomass concentration of 20 wt-% is reached in the concentrate (concentration factor 10). The energy demand for centrifuge operation is estimated at 4 $\mathrm{kWh/m^3_{feed}}$ [7]. It is assumed that 95% of the biomass is recovered [7].

For ORPs, due to the large amount of water in the cultivation medium, direct centrifugation would be too energy intensive. Instead, the cells are pre-concentrated by flocculation. A great deal of studies have investigated flocculation of microalgae by pH-shift, electrocoagulation, addition of metal salts, natural and synthetic cationic polymers, and bioflocculants [31]. For marine media in particular, the naturally high concentration of magnesium ions favors flocculation by pH-shift [32, 33, 34, 35, 36, 37, 38]. We assume that addition of 200 mg/L of slaked lime ($Ca(OH)_2$) results in the recovery of 95% of the biomass at a concentration factor of 40. To remove magnesium hydroxide from the precipitate [36, 39] and to neutralize the supernatant, HCl is subsequently added at a stoichiometric ratio of 2:1. The precipitate is then further dewatered in a centrifuge with parameters equal to the TLC scenario. Note that the additional dewatering step in the ORP configuration leads to higher biomass losses compared to the TLC configuration, leading in turn to an increased biomass demand per unit fuel.

In the ORP scenario, part of the supernatant from the harvest process is re-used for cultivation. In the TLC scenario, recycling is avoided to prevent salt accumulation. Blow-down in both scenarios is treated in a wastewater treatment plant.

3.4 Oil extraction and transport

To maximize oil yield, the algae cell walls are mechanically disrupted by a ball mill. Power consumption for this process is estimated at 0.06 kWh/L based on the data sheet of an industrial manufacturer [40]. After cell disruption, liquid hexane is applied to absorb the algae oil. The loaded hexane is removed and exposed to heat to release the oil. According to Frank et al. [41], oil recovery ratio, heat demand, electricity demand, and specific hexane loss of this process are 95%, 6.1 $\mathrm{MJ/kg_{oil}}$, 1.9 $\mathrm{MJ/kg_{oil}}$, and 5.2 $\mathrm{g/kg_{oil}}$, respectively.

The oil production rate of 190 kg/h is relatively small for processing in conventional refineries. To enable efficient operation, it is assumed that the output of multiple plants across Europe is collected and jointly processed in a dedicated refinery. Transport requirements for the collection process are approximated as follows: 50 km via truck to a collection point, 100 km via train to a harbor, 3 500 km via ship to the refinery.

3.5 Conversion, fuel transport & use

The algae oil is converted into so-called HEFA fuel (hydrotreated esters and fatty acids) to be used for aviation. The conversion process model is adopted from Zschocke [42]: Phospholipids and other impurities are removed prior to conversion by application of phosphoric acid (0.62 $\mathrm{g/kg_{oil}}$) and sodium hydroxide (1.9 $\mathrm{g/kg_{oil}}$). The clean oil is then hydroprocessed, removing heteroatoms and saturating the carbon bonds. Hydrocracking serves to increase the yield of middle distillates in the jet fuel range. Light fractions are consumed on-site to supply process energy (0.24 MJ//kg_{oil}) and hydrogen for hydroprocessing. The remaining fractions (mainly jet fuel, diesel, naphtha) total 1 MJ lower heating value by definition of the functional unit. Direct CO_2 emissions during the conversion process amount to 0.50 kg/kg_{oil}.

After conversion, the fuel fractions are transported 400 km via pipeline to a depot and then 50 km by truck to the end user. The end user burns the algal fuels, releasing CO_2 to the atmosphere. Note that because this CO_2 is originally derived from biomass (biomass → power plant flue gas → algae biomass → algal fuel combustion) and absorbed and re-emitted within a short time frame, it is regarded as climate neutral in accordance with the ILCD guidelines [43].

3.6 Residue valorization

Apart from oil, the hexane extraction process produces a wet biomass residue still containing significant amounts of carbon and energy. This residue is converted to biogas in an anaerobic digester. Accounting for the high feed salinity, a medium-to-low methane yield of 190 mL/g VS (volatile solids) is assumed [44]. The following further assumptions are based on expert interviews: The biogas consists of methane (60 vol.-%) and CO_2 (40 vol.-%). 2.0% of the produced methane leak to the atmosphere. Electricity is needed for pumping and mixing in the digester (0.40 MJ/kg VS). Heat is needed to maintain mesophilic conditions (0.23 MJ/kg wet). We assume that N and P contained in the digester feed remains in the digestate along with the C not turned into methane or CO_2. These offer a valuable nutrient supplement for algae cultivation. It is assumed that 50% of the contained C, N, and P is bio-available, displacing corresponding amounts of CO_2 and fertilizers.

The biogas is burnt in the previously mentioned CHP plant, producing heat, electricity, and CO_2. In this way, residue valorization supplies additional 0.24 MJ of useful heat and 0.20 MJ of electricity per unit fuel produced. As before, combustion CO_2 is captured and supplied to the cultivation plant.

4 Results and discussion

Figure 2 shows the life cycle climate impacts (GWP 100) of the TLC pathway, the ORP pathway, and the conventional baseline (petroleum kerosene). The TLC pathway has the lowest GHG intensity at 81 g CO_2e per MJ fuel lower heating value (LHV), followed by the fossil reference (84 g CO_2e) and the ORP pathway (94 g CO_2e). This result supports prior techno-economic analyses, which found that TLCs could outperform ORP technology [12, 24]. Compared to petroleum kerosene, the TLC pathway achieves a GHG reduction of 4%. However, these savings are too small to comply with the Renewable Energy

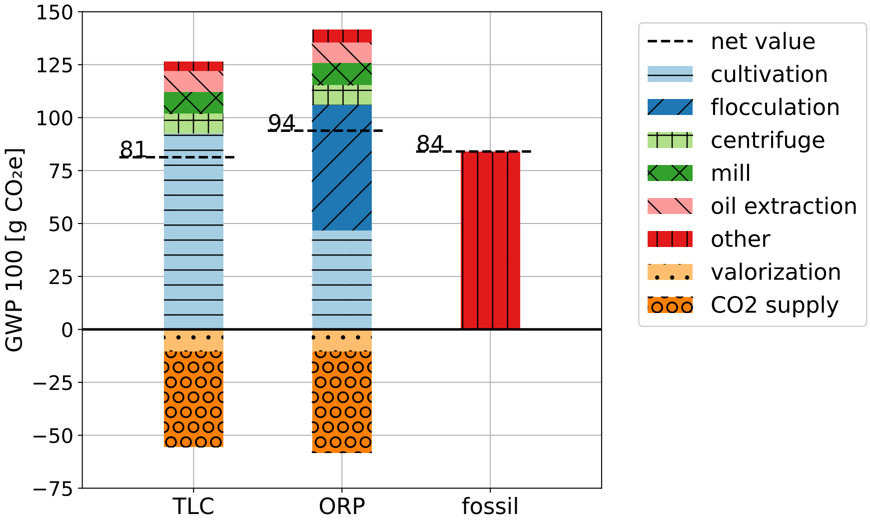

Fig. 2 Life cycle climate impact (GWP 100) of algal fuel from thin-layer cascade (TLC) cultivation, of algal fuel from open raceway pond (ORP) cultivation, and of conventional petroleum-based kerosene (fossil baseline)

Directive II (65% reduction for installations starting operation after 1 January 2021)[5]. Thus, both algal fuel pathways need improvement before they can contribute towards the goals of the Paris Agreement [3]. The following section shall highlight the largest emission sources in each pathway to direct future development efforts.

Our results indicate that, for both the TLC and the ORP pathway, inputs into the cultivation- and harvest stage dominate the life cycle climate impact. This finding is in line with previous studies on algae fuel production [9, 45, 10, 11]. Breaking down impacts of the TLC pathway, the largest contribution comes from seawater desalination (54 g CO_2e per MJ fuel LHV), mostly from energy consumed in the reverse osmosis process. This impact can be reduced by employing renewable energy or by supplying freshwater from a natural source. In the latter case, the impact on local water reserves must be evaluated critically. Without the contribution from reverse osmosis, the residual life cycle impact of TLC algal fuel production is 27 g CO_2e, indicating a potential to drive GHG emissions below the RED II threshold.

The second largest GHG contribution in the TLC pathway is power consumed for mixing (19 g CO_2e for operating the circulation pump during day and an air compressor at night). Although the nominal power demand per unit reactor area is higher for TLCs than ORPs, the biomass yield is proportionally higher, yielding a similar energy demand per unit biomass. Experimental power consumption measurements for large-scale TLC cultivation are unavailable at the time of writing. More generally, mixing power influences biomass yields by controlling the light-dark-cycles of the algal cells. Reducing the mixing power is thus expected to reduce biomass yields. Further research is necessary to define an optimum between power consumption and biomass production in TLCs.

The third largest GHG contribution in the TLC pathway is urea supply (16 g CO_2e). The role of fertilizers in the GHG balance of algae fuel production has been highlighted by previous studies [45, 11, 8, 9]. Its impact can be reduced by employing digestate recycling, as modeled in our study. If these reductions turn out insufficient, new nutrient sources (e.g. municipal or industrial wastewater) can be explored or existing synthetic fertilizer production can be improved (e.g. by employing green electricity and -hydrogen).

In the ORP model, cultivation impacts are dominated by paddle wheel operation (19 g CO_2e per MJ fuel LHV), followed by urea production (17 g CO_2e) and seawater replenishment (9 g CO_2e). The power demand for paddle wheel operation is low compared to other studies such as Doucha and Lívanský [12] and Acién Fernández et al. [24]. Applicable validation data are scarce as few large-scale ORP plants produce algal lipids in autotrophic growth mode. Again, mixing power and biomass yield are intertwined and further research is necessary to narrow the range of assumptions. For urea production, the same comments as for the TLC apply. For seawater replenishment, the pumping power follows eq. 5 and is most easily reduced by locating the cultivation plant as close as possible to the sea, as well as by reducing water losses. The latter are driven largely by evaporation. Collet et al. [11] suggest to cover open cultivation systems by greenhouses, thus reducing the impact of evaporation.

Apart from cultivation, flocculation presents a large contribution to the ORP GHG balance. Impacts are shared equally between HCl production (29 g CO_2e per MJ fuel lower heating value) and treatment of discarded culture medium (29 g CO_2e) whereas the impact of lime production and consumption is negligible. The HCl-related impact can be reduced if the leeching step is omitted, meaning that $Mg(OH)_2$ remains in the precipitate. This could, however, negatively affect anaerobic digester performance downstream, as its microbial consortia are sensitive to the concentration of alkaline earth metals [46]. Concerning the impact of blow-down treatment, it is proportional to the amount of blow-down and its pollution. The former is governed by eq. 4 and if blow-down were to be reduced, a freshwater source would become necessary with corresponding consequences for the GHG balance (see TLC impacts). For the latter, we chose an 'average' pollution (according to ecoinvent definition). The actual impact will depend not only on the degree of pollution but also on local discharge regulations and will thus vary from location to location.

5 Conclusion

Our study presents for the first time a life cycle climate impact assessment of aviation fuel produced from algal biomass cultivated in thin-layer cascades (TLCs). Our results support the notion of prior economic assessments that TLCs could offer an advantage over established open raceway technology

[12, 24]. Still, improvements are most likely necessary before TLC algal fuel can achieve the GHG savings required by the Renewable Energy Directive II [5]. Unlike ORPs, TLCs depend on a freshwater source to maintain stable salt concentrations in the culture medium. To avoid the high GHG impacts associated with seawater desalination, cultivation locations with nearby freshwater sources are preferable, where a low impact on natural reserves can be assured. Furthermore, our results indicate that electricity demand for water circulation presents a relevant contribution to the GHG footprint, although its magnitude is rather uncertain. Establishment of an empirical correlation between power consumption and biomass yield for various climatic conditions would help to reduce this uncertainty. Lastly, our results support the finding of previous studies that fertilizer consumption contributes significantly to the life cycle climate impact [45, 11, 8, 9]. Future experiments should thus clarify to which degree digestate recycling can reduce fertilizer demand. Our results indicate that a moderate nutrient recovery yields insufficient GHG savings and that other, climate-friendly nutrient sources must be explored.

Conflicts of interest

The authors declare no conflicts of interest.

Declarations

Funding

This work was supported financially by the Bavarian Ministry of Economic Affairs, Regional Development and Energy via grant number LABAY74C.

Conflicts of interest

The authors declare no conflicts of interest.

Availability of data and material

Not applicable.

Code availability

The following files are available free of charge in the supporting information:

- model_ORP.xlsx: ORP model
- model_TLC.xlsx: TLC model
- import_ORP.py: Python script to import ORP model into Brightway2

- import_TLC.py: Python script to import TLC model into Brightway2
- environment.yml: conda environment file (installs all necessary dependencies, incl. Brightway2)

Authors' contributions

Conceptualization: Christian H. Endres, Benjamin W. Portner, Daniel Garbe, Thomas Brück; Methodology: Christian H. Endres, Benjamin W. Portner; Formal analysis and investigation: Christian H. Endres, Benjamin W. Portner, Daniel Garbe, Thomas Brück; Writing - original draft preparation: Benjamin W. Portner; Writing - review and editing: Benjamin W. Portner, Christian H. Endres, Thomas Brück; Funding acquisition: Christian H. Endres; Resources: Thomas Brück, Daniel Garbe; Supervision: Christian H. Endres.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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